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# Morphology, composition, production, processing and applications of *Chlorella vulgaris*: A review

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ABSTRACT

Economic and technical problems related to the reduction of petroleum resources require the valorisation of renewable raw material. Recently, microalgae emerged as promising alternative feedstock that represents an enormous biodiversity with multiple benefits exceeding the potential of conventional agricultural feedstock. Thus, this comprehensive review article spots the light on one of the most interesting microalga *Chlorella vulgaris*. It assembles the history and a thorough description of its ultrastructure and composition according to growth conditions. The harvesting techniques are presented in relation to the novel algo-refinery concept, with their technological advancements and potential applications in the market.



**Contents**



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## 1. Introduction

Microalgae have an ancient history that left a footprint 3.4 billion years ago, when the oldest known microalga, belonging to the group of cyanobacteria, fossilised in rocks of Western Australia. Studies confirmed that until our days their structure remains unchanged and, no matter how primitive they are, they still represent rather complicated and expertly organised forms of life [1]. Nevertheless, other reports estimated that the actual time of evolution of cyanobacteria is thought to be closer to 2.7 billion years ago [2,3]. Hence, evolutionary biologists estimate that algae could be the ancestors of plants. Thus, through time algae gave rise to other marine plants and moved to the land during the Palaeozoic Age 450 millions years ago just like the scenario of animals moving from water onto land. However, evolutionists need to overcome multiple obstacles (danger of drying, feed, reproduction, and protection from oxygen) to definitely confirm this scenario complemented with more scientific evidence.

Like any other phytoplankton, microalgae have a nutritional value. The first to consume the blue green microalga were the Aztecs and other Mesoamericans, who used this biomass as an important food source [4]. Nowadays, these microscopic organisms are still consumed as food supplement such as *Chlorella vulgaris* and *Spirulina platensis* [5] and their products are also used for different purposes like dyes, pharmaceuticals, animal feed, aquaculture and cosmetics. For the last two decades, microalgae started to take a new course with increasing applications motivated by the depletion of fossil fuel reserves, the consequent increase in oil prices and the global warming concern. These dramatic thresholds are forcing the world to find global strategies for carbon dioxide mitigation by proposing alternative renewable feedstocks and intensifying researches on third-generation biofuels. In this context, microalgae are regarded nowadays as a promising sustainable energy resource due to their capacity to accumulate large quantities of lipids suitable for biodiesel production that performs much like petroleum fuel [6,7]. They also proved to be a source of products such as proteins, carbohydrates, pigments, vitamins and minerals [8]. In addition, microalgae capture sunlight and perform photosynthesis by producing approximately half of atmospheric oxygen on earth and absorbing massive amounts of carbon dioxide as a major feed. Therefore, growing them next to combustion power plants is of major importance due to their remarkable capacity to absorb carbon dioxide that they convert into potential biofuel, food, feed and highly added value components [9–14].

Microalgae can grow in both fresh and marine water as well as in almost every environmental condition on earth from frozen lands of Scandinavia to hot desert soils of the Sahara [15]. If production plants were installed in an intelligent way, microalgae would not compete with agricultural lands, there would be no conflict with food production [16] and especially would not cause deforestation.

Microalgae represent an enormous biodiversity from which about 40.000 are already described or analysed [17]. One of the most remarkable is the green eukaryotic microalga *C. vulgaris*, which belongs to the following scientific classification: Domain: Eukaryota, Kingdom: Protista, Divison: Chlorophyta, Class: Trebouxiophyceae, Order: Chlorellales, Family: Chlorellaceae, Genus: Chlorella, Specie: *Chlorella vulgaris*. Hence, Martinus Willem Beijerinck, a Dutch researcher, first discovered it in 1890 as the first microalga with a well-defined nucleus [18]. The name *Chlorella* comes from the Greek word *chloros* (Χλωρός), which means green, and the Latin suffix *ella* referring to its microscopic size. It is a unicellular microalga that grows in fresh water and has been present on earth since the pre-Cambrian period 2.5 billion years ago and since then its genetic integrity has remained constant [1]. By the early 1900s, *Chlorella* protein content ($>55\%$ dry weight) attracted the attention of German scientists as an unconventional food source. In the 1950s, the Carnegie Institution of Washington [19] took over the study and managed to grow this microalga on a large scale for $CO_2$ abatement. Nowadays, Japan is the world leader in consuming *Chlorella* and uses it for medical treatment [20,21] because it showed to have immune-modulating and anti-cancer properties [22–26]. After feeding it to rats, mice and rabbits in the form of powder, it showed protection properties against haematopoiesis [27] age-related diseases like cardiovascular diseases, hypertension and cataract; it lowers the risk of atherosclerosis and stimulates collagen synthesis for skin [28,29]. Furthermore, *C. vulgaris* is also capable of accumulating important amounts of lipids, especially after nitrogen starvation with a fatty acid profile suitable for biodiesel production [30,31].

The available reviews have focused so far on evaluating microalgae as an important source of lipids for biofuel production [32,33] and also explained in details the different production processes and harvesting techniques. The following review covers greater information about *C. vulgaris*, including not only production and harvesting techniques already conducted on this microalga, but also detailed information about its ultrastructure and chemical composition accompanied by cell wall breaking techniques and extraction processes. The last section focuses on the multiple applications and potential interests of this microalga in different areas and not only on the production of fatty compounds.

## 2. Morphology

*C. vulgaris* is a spherical microscopic cell with 2–10 μm diameter [33–35] and has many structural elements similar to plants (Fig. 1).

### 2.1. Cell wall

The rigidity preserves the integrity of the cell and is basically a protection against invaders and harsh environment. It varies according to each growth phase. During its early formation in its autosporangia, the newly formed cell wall remains fragile, forming a 2 nm thin electron-dense unilaminar layer [33,36]. The cell wall of the daughter cell gradually increases in thickness until it reaches 17–21 nm after maturation [33,35], where a microfibrillar layer is formed representing a chitosan-like layer composed of glucosamine [36,37], which accounts for its rigidity. In the mature stage, cell wall thickness and composition are not constant because they can change according to different growth and environmental conditions. Furthermore, some reports [38,39] explained the rigidity of the cell wall by focusing on the presence of a sporopollenin layer, even though it is generally accepted that *C. vulgaris* has a unilaminar cell wall that lacks sporopollenin, which is an extremely resistant polymerised carotenoid found on the cell wall of *Haematococcus pluvialis* [40] and *Chlorella fusca* [41]. However, a contradictory study conducted on *C. vulgaris* by Martinez et al. [42] reported the presence of sporopollenin by observing an outer trilaminar layer and by detecting resistant residues after being submitted to acetolysis.

### 2.2. Cytoplasm

It is the gel-like substance confined within the barrier of the cell membrane and it is composed of water, soluble proteins and minerals. It hosts the internal organelles of *C. vulgaris* such as mitochondria, a small nucleus, vacuoles [43], a single chloroplast and the Golgi body [44].

#### 2.2.1. Mitochondrion

Every mitochondrion contains some genetic materials, the respiratory apparatus and has a double-layer membrane; the outer membrane surrounds the whole organelle and is composed of an equal ratio of proteins and phospholipids. Nevertheless, the inner membrane is composed of thrice more proteins than phospholipids; it surrounds the internal space called the matrix, which contains the majority of mitochondrial proteins [44].

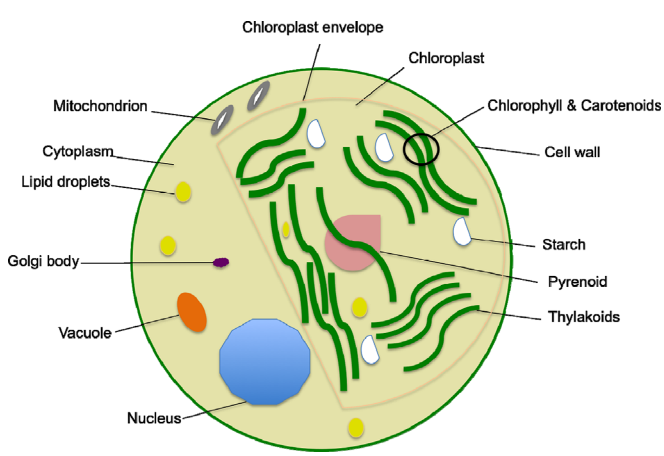



**Fig. 1.** Schematic ultrastructure of *C. vulgaris* representing different organelles.

#### 2.2.2. Chloroplast

*C. vulgaris* has a single chloroplast with a double enveloping membrane composed of phospholipids; the outer membrane is permeable to metabolites and ions, but the inner membrane has a more specific function on proteins transport. Starch granules, composed of amylose and amylopectin, can be formed inside the chloroplast, especially during unfavourable growth conditions. The pyrenoid contains high levels of ribulose-1,5-bisphosphate carboxylase oxygenase (RuBisCO) and is the centre of carbon dioxide fixation. The chloroplast also stores a cluster of fused thylakoids where the dominant pigment chlorophyll is synthesised masking the colour of other pigments such as lutein. During nitrogen stress, lipid globules mainly accumulate in the cytoplasm and the chloroplast [15,45].

## 3. Reproduction

*C. vulgaris* is a non-motile reproductive cell (autospore) that reproduces asexually and rapidly. Thus, within 24 h, one cell of *C. vulgaris* grown in optimal conditions multiplies by autosporulation, which is the most common asexual reproduction in algae. In this manner, four daughter cells having their own cell wall are formed inside the cell wall of the mother cell (Figs. 2 and 3) [33,35]. After maturation of these newly formed cells, the mother cell wall ruptures, allowing the liberation of the daughter cells and the remaining debris of the mother cell will be consumed as feed by the newly formed daughter cells.

## 4. Production

Annual production of *Chlorella* reached 2000 t (dry weight) in 2009, and the main producers are Japan, Germany and Taiwan [46]. This microalga has a rapid growth rate and responds to each set of growth condition by modifying the yield of a specific component. *C. vulgaris* is ideal for production because it is remarkably resistant against harsh conditions and invaders. On the one hand, lipid and starch contents increase and biomass productivity ceases or decreases [47] during unfavourable growth conditions such as nitrogen and phosphorus limitation, high $CO_2$ concentration, excessive exposure to light [30,48–50], excess of iron in the medium [51] or increase in temperature [52]. On the other hand, protein content increases during normal and managed growth conditions (nitrogen supplementation). Therefore, many growth techniques have been tested in order to voluntarily target biomass productivity, lipid, proteins, carbohydrates and pigments content.

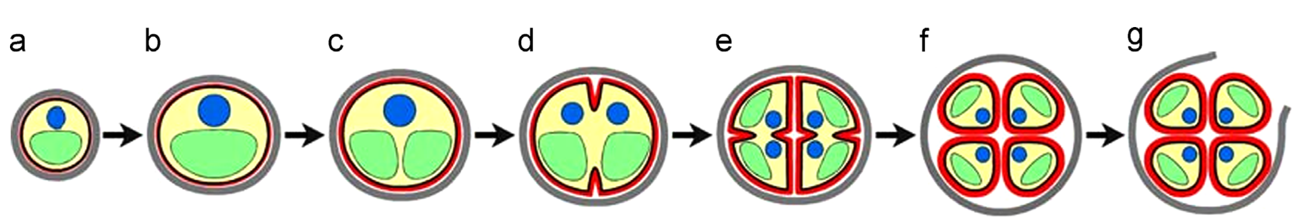



**Fig. 2.** Drawings showing the different phases of daughter cell-wall formation in *Chlorella vulgaris*: (a) early cell-growth phase; (b) late cell-growth phase; (c) chloroplast dividing phase; (d) early protoplast dividing phase; (e) late protoplast dividing phase; (f) daughter cells maturation phase and (g) hatching phase [35].

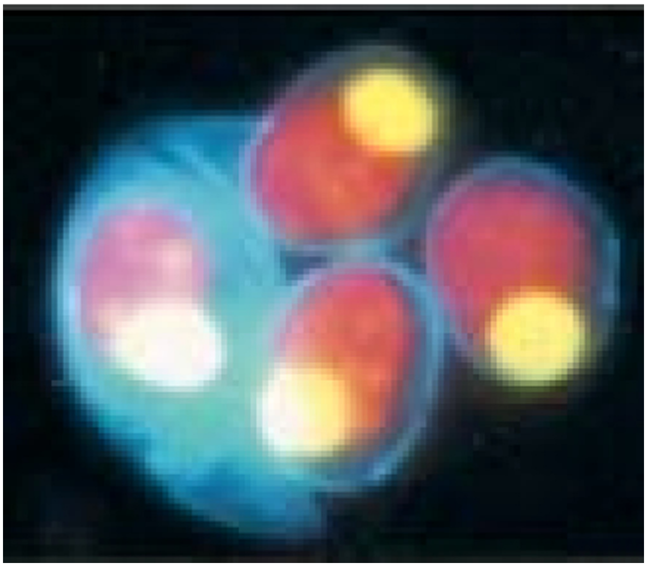


**Fig. 3.** Newly formed cells emerging outside the cell wall of the mother cell after hatching [33].

### 4.1. Autotrophic growth

#### 4.1.1. Open pond systems

Open ponds are the most common way of production and are the cheapest method for large-scale biomass production. These systems are categorised into natural waters (lakes, lagoons and ponds) or wastewater or artificial ponds or containers. They are usually built next to power plants or heavy industry with massive carbon dioxide discharge where the biomass absorbs nitrogen from the atmosphere in the form of $NO_x$. In order to allow easy exposure of all the cells to sunlight, especially at the end of the exponential growth phase, the optimal pond depth is 15–50 cm [46,52]. On the other hand, open pond systems have some limitations because they require a strict environmental control to avoid the risk of pollution, water evaporation, contaminants, invading bacteria and the risk of growth of other algae species. In addition, temperature differences due to seasonal change cannot be controlled and $CO_2$ concentration and excess exposure to sunlight are difficult to manage. Moreover, near the end of the exponential growth phase, some cells are not sufficiently exposed to sunlight because other cells floating near the surface cover them, leading to lower mass yields. Therefore, stirring of the medium is preferable and is currently practiced.

#### 4.1.2. Closed photo-bioreactor

This technology was implemented mainly to overcome some limiting factors in the open pond systems, thus growing the biomass in a managed environment (pH, light intensity, temperature, carbon dioxide concentration) to obtain higher cell concentration as well as products that are more suitable for the production of pure pharmaceuticals, nutraceuticals and cosmetics. In addition, these systems are more appropriate for sensitive strains that cannot compete and grow in harsh environment. Feeding the biomass with $CO_2$ comes by bubbling the tubes. Fluorescent lights are used in case the tubes are not or not sufficiently exposed to sunlight. The tubes are generally 20 cm or less in diameter [32] and the thickness of their transparent walls is few millimetres, allowing appropriate light absorption. Hence, multiple designs have been used and tested: flat-plate photo-bioreactor [53,54], tubular photo-bioreactor [55] and column photo-bioreactor [56]. Degen et al. [57] achieved 0.11 $g\,L^{-1}\,h^{-1}$ dry biomass productivity after growing the cells of *C. vulgaris* in a flat panel airlift photobioreactor under continuous illumination (980 $\mu E\,m^{-2}\,s^{-1}$). Nonetheless, the main disadvantages of a closed system are the cost of the sophisticated construction, small illumination area and sterilising costs [58].

### 4.2. Heterotrophic growth

This technique does not require light and the biomass is fed with organic carbon source. Thus, microalgae are grown in a stirred tank bioreactor or fermenter where a higher degree of growth are expected as well as low harvesting cost due to the higher dry biomass productivity achieved (up to 0.25 $g\,L^{-1}\,d^{-1}$) and high accumulation of different components such as lipids 22–54 $mg\,L^{-1}\,d^{-1}$ [42,59,60]. The carbon sources used for *C. vulgaris* are glucose, acetate, glycerol and glutamate with maximum specific growth rate obtained with glucose. Nevertheless, the major disadvantage of this system is the price and availability of sugars, which compete with feedstocks for other uses such as food and biofuel productions.

### 4.3. Mixotrophic growth

*C. vulgaris* is capable of combining both autotrophic and heterotrophic techniques by performing photosynthesis as well as ingesting organic materials such as glucose, which is the most appropriate for *C. vulgaris* [59–63]. Hence, the cells are not strictly dependent on light or organic substrate to grow. This technique competes favourably with autotrophic systems and according to Yeh and Chang [63] mixotrophic conditions showed high dry biomass productivity (2–5 $g\,L^{-1}\,d^{-1}$) and lipids productivity (67–144 $mg\,L^{-1}\,d^{-1}$). The main advantages of mixotrophic metabolism are limiting the impact of biomass loss during dark respiration and reducing the amount of organic substrates used for growing the biomass.

### 4.4. Other growth techniques

Growth of *C. vulgaris* can take an additional dimension by co-immobilising it with plant growing bacterium *Azospirillum brasilense* in alginate beads [64,65]. This technique has been extrapolated to *C. vulgaris* and other microalgae from the hypothesis that *A. brasilense* promotes terrestrial plant growth performance by interfering with the host plant hormonal metabolism and provides $O_2$ for the bacteria to biodegrade pollutants and then the microalga consumes $CO_2$ released from bacterial respiration [66]. Consequently, depending on the strain of *C. vulgaris* [67] this technique has an impact on prolonging its life span, enhancing biomass production, increasing cell size (62% larger) and accumulating pigments and lipids. Simultaneously, uptake of zinc, cadmium, phosphorus, nitrogen and other heavy metals from wastewater increases. On the other hand, growing *C. vulgaris* with its associative bacterium *Phyllobacterium myrsinacearum* also has a different impact by ceasing its growth or cell death [68]. Furthermore, mixing and shear stress have an effect on increasing the photosynthetic activity and growth of *C. vulgaris*. Thus, optimal conditions (tip speed of 126 $cm\,s^{-1}$ and friction velocity 2.06 $cm\,s^{-1}$) increased the photosynthetic activity by 4–5% with 48–71% stronger growth compared to null tip speed or friction velocity. Nevertheless, higher tip speed and friction velocity decreased both photosynthetic activity and growth to the value of the unstirred condition and even lower [69].

### 4.5. Harvesting

#### 4.5.1. Centrifugation

This process contributes to 20–30% of the total biomass production cost [55]. The most common harvesting technique for *C. vulgaris* is centrifugation (5000 rpm, 15 min) [30,70] because it is highly efficient (95% recovery), not time consuming, and treats large volumes. In addition, the morphology of *C. vulgaris* permits high centrifugal stress without damaging its structure during the process. Other techniques are also applied such as flocculation, flotation and filtration or by combining two techniques to maximise recovery of the biomass.

#### 4.5.2. Flocculation

During the exponential growth phase, the algal cells have high negative surface charge and are difficult to neutralise, and thus the cells remain dispersed. After reaching the stationary or the declining phase, the negative charge decreases, allowing the cells to aggregate and to form lumps, thereby resulting in a process called auto-flocculation. This phenomenon is associated with elevated pH due to $CO_2$, nitrate and phosphate assimilation [71]. Moreover, auto-flocculation can occur by interactions between algae and bacteria or excreted organic molecules or by simply cutting $CO_2$ supply; this method is less expensive but time-consuming. In general, culture of microalgae is very stable and auto-flocculation probability is negligible and sometimes misleading. In order to accelerate coagulation, it is necessary to increase the pH by adding a base. The most effective is sodium hydroxide, which induces more than 90% flocculation at pH 11 and requires less quantity (9 mg of NaOH per gram of dry biomass) [71,72]. But on an industrial scale, lime seems to be the most cost-efficient. This mechanism is associated with $Mg^{2+}$ from hydrolysed Mg $(OH)_2$, which precipitates attracting with it the negatively charged microalgal cells. Chitosan is also an interesting flocculating agent [73], which showed maximum efficiency at pH 7 with 90% microalgal recovery. Further on, using bioflocculants like *Paenibacillus* sp. with the presence of a co-flocculant ($CaCl_2$) also showed an efficient flocculation (83%) at pH 11 [74]. Flocculation is sometimes considered as a pre-harvesting step in order to facilitate or complement other harvesting methods like centrifugation or filtration [75,76].

#### 4.5.3. Flotation

To our knowledge, there is very limited evidence of its feasibility, but this method consists of trapping the cells using dispersed micro-air bubbles. Flotation can also occur naturally when the lipid content in microalgae increases. Cheng et al. [77] induced effective flotation on *C. vulgaris* by using dispersed ozone gas (0.05 mg $g^{-1}$ biomass). Thus, unlike flocculation, this method does not require synthetic chemicals, but its economic viability is not yet known, especially on an industrial scale.

#### 4.5.4. Filtration

This method involves continuous passing of the broth with the microalga across a filter on which algal cells will concentrate constantly until it reaches a certain thickness. Due to the small size of *C. vulgaris*, conventional filtration is not an adequate method to be applied. Instead, ultrafiltration or microfiltration is more efficient. Fouling generated by soluble compounds like exopolysaccharides of some microalgae such as *Porphyridium* is one of the major limitations during the ultrafiltration process, but with *Chlorella* this phenomenon is negligible, and thus its structure provides more important permeation flux without the need of an additional unit operation like swirling while filtering [78,79]. Moreover, microfiltration and ultrafiltration are affected by different parameters such as filter type; transmembrane pressure, flow velocity, turbulent cross-flow and growth phase, and therefore a compromise that takes into consideration these parameters should be made. Furthermore, they can be accompanied by another harvesting technique (flotation or flocculation) that improves the process [75,76,80].

## 5. Primary composition

### 5.1. Proteins

Proteins are of central importance in the chemistry and composition of microalgae. They are involved in capital roles such as growth, repair and maintenance of the cell as well as serving as cellular motors, chemical messengers, regulators of cellular activities and defence against foreign invaders [44].

Total proteins content in mature *C. vulgaris* represents 42–58% of biomass dry weight [81–85], and varies according to growth conditions. Proteins have multiple roles, and almost 20% of the total proteins are bound to the cell wall, more than 50% are internal and 30% migrate in and out of the cell [86]. Their molecular weight revealed by SDS-PAGE comprises between 12 and 120 kDa, with the majority between 39 and 75 kDa after growing *C. vulgaris* under autotrophic or heterotrophic conditions. Nevertheless a higher intensity peak is observed for cells grown in autotrophic conditions [82,87].

Protein nutritional quality is determined by its amino acid profile [81,88], and like the majority of microalgae, the amino acid profile of *C. vulgaris* compares favourably and even better with the standard profile for human nutrition proposed by World Health Organisation (WHO) and Food and Agricultural Organisation (FAO), because the cells of *C. vulgaris* synthesise essential and non-essential amino acids (Table 1). Furthermore, regardless of the extraction procedure, *C. vulgaris* proteins showed excellent emulsifying capacity [89] that is comparable and even better than the commercial ingredients. Results showed that the emulsifying capacity of *C. vulgaris* proteins extracted at $pH=7$ reached $3090 \pm 50$ mL oil/g protein with a stability of $79 \pm 1$%. Therefore, proteins of *C. vulgaris* open the gate for additional valorisation options of this microalga in the market, especially in the food sector.

Protein extraction is technically the same for all microalgae and is mainly conducted by solubilisation of proteins in alkaline solution [83,90,91]. Further purification can be followed by precipitating the solubilised proteins with trichloroacetic acid (25% TCA) [92,93] or hydrochloric acid (0.1 N HCl) [94]. Another separation method could be applied by means of ultrafiltration. Indeed, this method is usually applied for harvesting the cells but considering the study conducted by Safi et al. [95], a two-stage ultrafiltration process was applied on the aqueous extract of *Tetraselmis suecica* containing solubilised molecules (starch, proteins and low molecular weight polysaccharides). The first phase of the process completely retained starch molecules, and then the second phase completely retained proteins, allowing only small polysaccharides to be present in the filtrate of the second phase of the process. This process could be extrapolated to *C. vulgaris* with minor modifications of the cut-off of the ultrafiltration membranes [95].

Quantification is carried out by elemental analysis, Kjeldahl, Lowry assay, Bradford assay or the dye binding method. However, the first two analyses take into consideration total nitrogen present in the microalga, and multiplying it by the standard nitrogen to protein conversion factor (NTP) 6.25 may lead to overestimation or underestimation of the true protein quantity. Therefore, several studies calculated from an amino acid profile

**Table 1**
Amino acid profile of *Chlorella vulgaris* compared to other resources expressed in grams per 100 g of protein.

| Amino acids | *C. vulgaris*[b] | *C. vulgaris*[a] | *C. vulgaris*[c] | Recommendation from FAO/WHO[b] | Eggs[b] | Soya[b] |
|---|---|---|---|---|---|---|
| **Aspartic acid** | 9.30 | 10.94 | 9.80 | N/A | 11.00 | 1.30 |
| **Threonine** | 5.30 | 6.09 | 5.15 | 4.00 | 5.00 | 4.00 |
| **Serine** | 5.80 | 7.77 | 4.32 | N/A | 6.90 | 5.80 |
| **Glutamic acid** | 13.70 | 9.08 | 12.66 | N/A | 12.60 | 19.00 |
| **Glycine** | 6.30 | 8.60 | 6.07 | N/A | 4.20 | 4.50 |
| **Alanine** | 9.40 | 10.90 | 8.33 | N/A | n.d | 5.00 |
| **Cysteine** | n.d | 0.19 | 1.28 | 3.50 | 2.30 | 1.90 |
| **Valine** | 7.00 | 3.09 | 6.61 | 5.00 | 7.20 | 5.30 |
| **Methionine** | 1.30 | 0.65 | 1.24 | N/A | 3.20 | 1.30 |
| **Isoleucine** | 3.20 | 0.09 | 4.44 | 4.00 | 6.60 | 5.30 |
| **Leucine** | 9.5 | 7.49 | 9.38 | 7.00 | 7.00 | 7.70 |
| **Tyrosine** | 2.80 | 8.44 | 3.14 | 6.00 | 4.20 | 3.20 |
| **Phenylalanine** | 5.50 | 5.81 | 5.51 | N/A | 5.80 | 5.00 |
| **Histidine** | 2.00 | 1.25 | 1.97 | N/A | 2.40 | 2.60 |
| **Lysine** | 6.40 | 6.83 | 6.68 | 5.50 | 5.30 | 6.40 |
| **Arginine** | 6.90 | 7.38 | 6.22 | N/A | 6.20 | 7.40 |
| **Tryptophan** | n.d | 2.21 | 2.30 | 1.00 | 1.70 | 1.40 |
| **Ornithine** | n.d | 0.13 | n.d | N/A | n.d | n.d |
| **Proline** | 5.00 | 2.97 | 4.90 | N/A | 4.20 | 5.30 |

n.d: not detected; N/A: not available.

[a] [83].
[b] [192,193].
[c] [194].

recommended a new NTP lower than the standard 6.25 [96–100]. Nevertheless, a study conducted by Safi et al. [83] correlated the evaluation of the NTP to the rigidity of the cell wall by evaluating the NTP of five crude microalgae including *C. vulgaris* and their protein extract, and concluded that no universal conversion factor could be recommended for multiple reasons such as cell wall rigidity, growth conditions, growth media and environmental uncertainty. Gonzalez-Lopez et al. [97] determined the NTP using a different technique that correlates protein content (Lowry assay) to total nitrogen content (Kjeldahl and elemental analysis) and also estimated that the Kjeldahl method correlates better with the Lowry assay. In addition, Servaites et al. [84] quantified proteins of 12 different microalgae including *C. vulgaris* by staining the protein isolate with Coomassie brilliant blue R-250 (CBB) on a paper and then eluting the remaining stained proteins in 1% sodium dodecyl sulphate (SDS) followed by measuring the absorbance at 600 nm. This method gave almost similar results compared to the Dumas method. On the other hand, the colorimetric method of Lowry [101] was also considered as one of the most accurate methods to quantify proteins [102], but with time this method showed to only quantify hydro-soluble proteins [83,88,101–105], which represents the major part of proteins. The Lowry assay is more acceptable than the Bradford assay because the latter does not react with all the amino acids present in the extract, thus giving lower protein concentrations [92].

### *5.2. Lipids*

Lipids are a heterogeneous group of compounds that are defined not by their structure but rather by the fact that they are soluble in non-polar solvents and relatively insoluble in water [90]. During optimal growth conditions *C. vulgaris* can reach 5–40% lipids per dry weight of biomass [81], and are mainly composed of glycolipids, waxes, hydrocarbons, phospholipids, and small amounts of free fatty acids [15,17]. These components are synthesised by the chloroplast and also located on the cell wall and on membranes of organelles (chloroplast and mitochondrion membranes). Nevertheless, during unfavourable growth conditions, lipids content (mainly composed of triacyglycerols) can reach 58% [8,81,106]. Unlike other lipids, triacylglycerols do not perform a structural role but instead accumulate as dense storage lipid droplets in the cytoplasm and in the inter-thylakoid space of the chloroplast [17].

Liu et al. [51] optimised a method that detects the accumulation of lipid droplets inside the cells of *C. vulgaris* after each growth phase. The method relies on staining the cells with Nile red dye and then observing the accumulation of lipids with fluorescence microscope by emitting blue light that reveals the lipid droplets, especially neutral lipids. This technique showed a correlation between the quantity of neutral lipids accumulated and fluorescence intensity. However, according to Chen et al. [107] without cell disruption, this method could be ineffective due to the presence of a thick cell wall of some microalgae that can prevent complete access of the reagent inside the cell. Thus, cell disruption is a necessity to prevent wrong measurements and quantification.

The extraction process of total lipids from *C. vulgaris* is generally conducted by the method of Bligh and Dyer (a mixture of chloroform and methanol), or by hexane, or petroleum ether [31,49,51,58,108–110]. Quantification of total lipids is conducted gravimetrically after evaporating the extracting solvent; in addition, column chromatography is carried out in order to separate different lipid constituents followed by evaporating the solvent and then weighing the remaining lipid extract [111]. Indeed, these solvents are not used on an industrial scale because they are harmful for the environment, toxic, highly flammable and contaminate the extract [109]. Total lipids are composed of three major fractions phospholipids (PL), glycolipids (GL) and neutral lipids (NL). These fractions are fractionated by sequential elution of chloroform and acetic acid for NL, acetone and methanol for GL, and methanol for PL recovery [111]. Supercritical carbon dioxide (SC-$CO_2$) extraction has been identified as an alternative for a greener extraction since it gives pure extracts free of contamination. Moreover, in order to increase the yield of extraction, a co-solvent to SC-$CO_2$ such as ethanol can be used or a preliminary cell disruption technique can be performed [112]. It is noteworthy that the addition of ethanol increases the extraction yield of total lipophilic molecules (lipids and pigments), but it could also bypass the energetic yet efficient cell disruption technique, and therefore the production cost could be significantly reduced [113].

The fatty acid profile changes with respect to growth conditions and is suitable for different applications. For instance, according to Yeh and Chang [63], the fatty acid profile of *C. vulgaris* grown under mixotrophic growth conditions can accumulate 60–68% saturated and monounsaturated fatty acids composed of palmitic acid C16:0, stearic acid C18:0 fatty acids, palmitoleic acid C16:1 and oleic acid C18:1 [31]. Such a profile is more suitable for biodiesel production [114]. On the contrary, if it is grown under favourable growth conditions, its fatty acid profile is unsuitable for biodiesel [106] but more suitable for nutritional uses because it is more concentrated in polyunsaturated fatty acids such as linoleic acid C18:2, linolenic acid C18:3, and eicosapentaenoic acid C20:5 [107].

### 5.3. Carbohydrates

Carbohydrates represent a group of reducing sugars and polysaccharides such as starch and cellulose. Starch is the most abundant polysaccharide in *C. vulgaris*. It is generally located in the chloroplast and is composed of amylose and amylopectin, and together with sugars they serve as energy storage for the cells. Cellulose is a structural polysaccharide with high resistance, which is located on the cell wall of *C. vulgaris* as a protective fibrous barrier. In addition, one of the most important polysaccharides present in *C. vulgaris* is the $\beta 1 \rightarrow 3$ glucan [115], which has multiple health and nutritional benefits.

Total carbohydrates are generally quantified by the sulphuric–phenol method [116,117], yielding simple sugars after hydrolysis at 110 °C, then quantification of the latter by HPLC (especially HPIC). Starch quantification is much better using the enzymatic method compared to the acidic method [118,119]. During nitrogen limitation, total carbohydrates can reach 12–55% dry weight.[120,121].

Moreover, *C. vulgaris* has a remarkably robust cell wall [122], mainly composed of a chitosan like layer, cellulose, hemicellulose, proteins, lipids and minerals [123–125].

The sugar composition (Table 2) of the cell wall is a mixture of rhamnose, galactose, glucose, xylose, arabinose and mannose [126–130], rhamnose being the dominant sugar [128,131,132].

### 5.4. Pigments

The most abundant pigment in *C. vulgaris* is chlorophyll, which can reach 1–2% dry weight and is situated in the thylakoids. *C. vulgaris* also contains important amounts of carotenoids (Table 3) that act as accessory pigments by catching light; β-carotene for instance is associated with the lipid droplets in the chloroplast, and primary carotenoids are associated with chlorophyll in thylakoids where they trap light energy and transfer it into the photosystem. However, as in terrestrial plants, some pigments act as photoprotectors by protecting chlorophyll molecules from degradation and bleaching during strong exposure to radiation and oxygen [44].

These pigments have multiple therapeutic properties, such as antioxidant activities [133], protective effect against retina degeneration [134,135], regulating blood cholesterol, prevention from chronic diseases (cardiovascular and colon cancer) and fortifying the immune system [136,137]. Pheophytins are biochemically similar to chlorophyll but lacking $Mg^{++}$ ion; they can form after chlorophyll degradation during the growth of microalgal cells or during harsh extraction conditions. In addition, these pigments are lipophilic and their extraction is generally associated with lipid extraction.

Many studies worked on optimising the extraction process of pigments using solvents (dimethyl formamide, dichloromethane, acetone, hexane, and ethanol), soxhlet, ultrasound-assisted extraction [70,138–141], and pressurised liquid extraction (PLE) that showed useful simultaneous extraction of carotenoids and chlorophyll, and also minimised the formation of pheophytins [70,142] at high temperature ($>110$ °C). Moreover, SC-$CO_2$ extraction was also carried out to enhance carotenoids recoveries, and the best conditions were 35 MPa and 40–55 °C on crushed cells, and under these conditions the extract was golden and limpid unlike solvents extraction; thus by using SC-$CO_2$, higher selectivity can be achieved [139,142]. This hypothesis was confirmed by Kitada et al. [20], using different optimum conditions (50 MPa and 80 °C) because the study was conducted on whole cells; thus stronger conditions were required. In addition, co-solvent such as 5% ethanol has been added as a booster to increase the extraction yield. Analyses and quantification of pigments are conducted by high performance liquid chromatography (HPLC) and spectrophotometry using specific equations [143] or by plotting the calibration curve for each pigment.

**Table 2**
Simple sugars composition of the cell wall polysaccharides [128].

| Neutral sugars | Percentage (%) |
|---|---|
| Rhamnose | 45–54 |
| Arabinose | 2–9 |
| Xylose | 7–19 |
| Mannose | 2–7 |
| Galactose | 14–26 |
| Glucose | 1–4 |

**Table 3**
Potential pigments content in *C. vulgaris* under different growth conditions.

| Pigments | μg g$^{-1}$ (dw) | References |
|---|---|---|
| β-Carotene | 7–12,000 | [20,65,70,139,170] |
| Astaxanthin | 550,000 | [170,195,196] |
| Cantaxanthin | 362,000 | [139,140,170,195] |
| Lutein | 52–3830 | [20,65] [67,70] [139,170] |
| Chlorophyll-*a* | 250–9630 | [65] [20,67] [68,139] |
| Chlorophyll-*b* | 72–5770 | [65] [20,67] [70,139] |
| Pheophytin-*a* | 2310–5640 | [70] |
| Pheophytin-*b* | N/A | [70] |
| Violoxanthin | 10–37 | [65] [67] |

N/A: not available.

### 5.5. Minerals and vitamins

Minerals are determined after incinerating the biomass and then analysis by atomic absorption spectrophotometry (Table 4). They play important functional roles in humans [44]. For instance, potassium cation is principal for human nutrition; it is associated with intracellular fluid balance, carbohydrate metabolism, protein synthesis and nerve impulses. In addition, it is used as chemical fertilizer in agriculture in the form of chloride (KCl), sulphate ($K_2SO_4$) or nitrate ($KNO_3$). Magnesium is important in maintaining normal and constant nervous activity and muscle contraction; hence magnesium deficiency in human organism can lead to depression and symptoms of suicidal behaviour. Zinc is an essential component of enzymes, which participates in many metabolic processes including synthesis of carbohydrates, lipids and proteins and it is also a cofactor of the superoxide dismutase enzyme, which is involved in the protection against oxidative processes and reducing the severity of strong diarrhoea.

**Table 4**
Minerals profile of *C. vulgaris*.

| Minerals | Mineral content (g 100 g$^{-1}$) | | |
|---|---|---|---|
| | Maruyama et al. [203] | Tokusoglu and Unal [197] | Panahi et al. [198] |
| Microelements | | | |
| Na | N/A | 1.35 | N/A |
| K | 1.13 | 0.05 | 2.15 |
| Ca | 0.16 | 0.59 | 0.27 |
| Mg | 0.36 | 0.34 | 0.44 |
| P | N/A | 1.76 | 0.96 |
| Macroelements | | | |
| Cr | N/A | tr | tr |
| Cu | N/A | tr | 0.19 |
| Zn | N/A | tr | 0.55 |
| Mn | N/A | tr | 0.40 |
| Se | N/A | tr | N/A |
| I | N/A | N/A | 0.13 |
| Fe | 0.20 | 0.26 | 0.68 |

tr: traces; N/A: not available.

**Table 5**
Vitamins profile of *C. vulgaris*.

| Vitamins | Content (mg 100 g$^{-1}$) | | |
|---|---|---|---|
| | Maruyama et al. [203] | Yeh et al. [114] | Panahi et al. [198] |
| B1 (Thiamine) | 2.4 | N/A | 1.5 |
| B2 (Riboflavin) | 6.0 | N/A | 4.8 |
| B3 (Niacin) | N/A | N/A | 23.8 |
| B5 (Pantothenic acid) | N/A | N/A | 1.3 |
| B6 (Pyridoxine) | 1.0 | N/A | 1.7 |
| B7 (Biotin) | N/A | N/A | 191.6 |
| B9 (Folic acid) | N/A | N/A | 26.9 |
| B12 (Cobalamin) | tr | N/A | 125.9 |
| C (Ascorbic acid) | 100.0 | 39.0 | 15.6 |
| E (Tocopherol) | 20.0 | 2787.0 | N/A |
| A (Retinol) | N/A | 13.2 | N/A |

tr: traces; N/A: not available.

Vitamins are classified as water-soluble (C and B) and fat-soluble (A, D, E, and K). *C. vulgaris* has an important vitamin profile (Table 5) that are key elements for cell growth and differentiation in the human body (Vitamin A), and have antioxidant activity that acts as radical scavenger together with improving blood circulation and controlling muscle functions (Vitamins E and C) [144]. Vitamin B complex occupies the largest number in living organisms and is a major actor for enzymes activity in metabolism [145], promotes red blood cells growth, reduces the risk of pancreatic cancer, and maintains healthy skin, hair and muscles. Vitamins profile is sensitive to growth conditions; thus the best concentration was achieved after 24 h autotrophic growth with 10% $CO_2$, but during heterotrophic conditions vitamins content was higher than autotrophic due to the presence of glucose in the medium and used as carbon source to produce organic compounds [87]. Another possible explanation for the high content of vitamins may be the alterations in the ultrastructure of the photosynthetic apparatus which were found to be associated with changes in cellular components [146].

## 6. Cell disruption techniques

*C. vulgaris* has a resistant cell wall, which is a major barrier for digestibility and extraction process of all internal components. Breaking the cell wall is an important challenge and a costly unit operation. Multiple techniques have been carried out on *C. vulgaris* (Table 6). Cooling the system during mechanical cell breaking is always required because the high-energy input overheats the broken microalga and jeopardises the integrity of target components by damaging or oxidising them. Enzymatic treatment is a promising technique that requires a deep understanding of the ultrastructure and composition of the cell wall in order to select the appropriate enzyme and to reduce the enzyme concentration required to hydrolyse the cell wall. According to Lee et al. [108] and Zheng et al. [31] the best cell disruption techniques with 30% dry weight lipid recovery of *C. vulgaris* grown under autotrophic conditions were autoclaving, microwave, enzymatic and grinding with liquid nitrogen. Nonetheless, the quality of the target molecules is susceptible to be different with respect to the cell disruption method applied. Thus, the amino acid profile of proteins obtained after conducting an alkaline treatment on *C. vulgaris* is different from the amino acid profile obtained after high-pressure homogenisation [147].

The success of cell disruption techniques is generally assessed by conducting microscopic observations or by comparing the extracted yield of a component before and after applying the cell disruption.

## 7. Applications and potential interests

### 7.1. Biofuels

Dependency on energy sources is growing faster, especially with the exponential increase in demand, which is leading to more dramatic consequences for the environment. Third generation biofuel form algae or microalgae is considered as one of the alternatives to current biofuel crops such as soybean, corn, rapeseed and lignocellulosic feedstocks because it does not compete with food and does not require arable lands to grow [16]. However, biofuel from microalgae is promising in the long term because it is now accepted that the production cost is still high and cannot yet compete with conventional fuel. But it competes favourably with crops by their potential of producing 10–20 times more oil [148] within a shorter period of time. As mentioned previously, *C. vulgaris* has the potential to accumulate high amounts of lipids, especially while growing it under mixotrophic conditions. Its fatty acid profile showed to be suitable for biodiesel production with an oxidative stability after transforming it to biodiesel, and has properties [149] that comply with the US Standard (ASTM 6751), European Standard (EN 14214), Brazilian National Petroleum Agency (ANP 255) and Australian Standard for biodiesel [150] and also compared favourably with (ASTM and EN) an Indian biodiesel standard [61]. After lipid extraction the remaining residue is rich in proteins, carbohydrates and minor amounts of lipids. Thus, Wang et al. [149] applied fast pyrolysis on *C. vulgaris* remnants using an atmospheric-pressure fluidised bed reactor at 500 °C and obtained bio-oil and biochar representing 94% of energy recovery from the remnant, without forgetting the small amount of biogas recovered. However, the quality of bio-oil was poor due to the presence of nitrogen in significant amounts (12.8% dry weight). Besides, *C. vulgaris* has high starch content and algal starch proved to be a good source for bioethanol production. Hirano et al. [151] extracted starch from *C. vulgaris* and achieved 65% ethanol-conversion rate after saccharification and fermentation with yeast. Hydrothermal liquefaction is another alternative route for biofuel production from microalgae. It involves the reaction of biomass in water at high temperature with or without the presence of a catalyst to obtain bio-crude [152]. The main advantage of this method is that it improved 10–15% the energetic

**Table 6**
Different cell disruption techniques carried out on *C. vulgaris*.

| Cell disruption | Time | Experimental set-up | References |
|---|---|---|---|
| **Acid treatment** | 25 min | Hot $Ac_2O+H_2SO_4$ (9:1, v-v) | [70] |
| **Alkaline treatment** | 60 min | 2 N NaOH | [83] |
| **Autoclaving** | 5 min | 125 °C + 1.5 MPa | [106] |
| **Bead milling** | 20 min | Beads: 0.4–0.6 mm<br>Rotational speed: 1500 rpm | [31] |
| | 5 min | Beads: 0.1 mm,<br>Rotational speed: 2800 rpm | [106] |
| | 2 min | Beads: 1 mm | [59] |
| **Electroporation** | N/A | Electric field: 3 kV/cm<br>Electrode: 2 cm | [73] |
| **Enzymatic lysis** | 60 min | Snailase (5 mg $L^{-1}$), 37 °C | [31] |
| | 10 h | Cellulase or lysozyme (5 mg $L^{-1}$), 55 °C | |
| | N/A | 4% Cellulase + 1% others (w/v)<br>25 mM sodium phosphate buffer<br>pH 7.0<br>0.5 M mannitol | [199] |
| | 10 h | 4% Cellulase + 1% macerozyme R10 + 1% pectinase (w/v)<br>pH 6.0<br>25 mM phosphate buffer<br>0.6 M sorbitol/mannitol (1:1) | [90] |
| | 24 h | Cellulase 0.5 mg L<br>0.5 M mannitol | [200] |
| **French press** | N/A | 138 MPa | [201] |
| | N/A | N/A | [78] |
| **Manual grinding** | 1–10 min | With liquid nitrogen or quartz | [31] |
| | N/A | With dry ice | [169] |
| **High pressure homogeniser** | N/A | N/A | [202] |
| **Microwaves** | 5 min | 100 °C, 2450 MHz | [31,106] |
| | 5 min | 40–50 °C, 2450 MHz | [107] |
| **Osmotic shock** | 48 h | 10% NaCl | [106] |
| | 60 min | 2 N NaOH | [83] |
| **Ultra-sonication** | 6 min | 10 W | [84] |
| | 20 min | 600 W | [31] |
| | 5 min | 10 kHz | [106] |
| | 15–60 min | N/A | [50] |

N/A: not available.

**Table 7**
Cumulative energy demand and energy production associated with the production of 1 MJ of biodiesel from *C. vulgaris* [159].

| Oil extraction | Nitrogen for culture | Energy production (MJ) | Cumulative energy demand (MJ) | Yield (MJ) |
|---|---|---|---|---|
| Dry | Sufficient | 2.7 | 5.29 | −2.59 |
| Wet | Sufficient | 3.84 | 3.99 | −0.15 |
| Dry | Low | 1.57 | 2.32 | −0.75 |
| Wet | Low | 2.23 | 1.66 | 0.57 |

value of *C. vulgaris* by acting on the whole biomass, suggesting that oil is also derived from carbohydrates and proteins [153], and thus no need to stress the microalgae to increase lipid content. Hence, the best conditions applied on *C. vulgaris* in a batch reactor were 300–350 °C, with 150–200 bar in water or with the presence of an organic acid or heterogeneous catalysts, and the results indicate that bio-oil formation follows the trend lipids > proteins > carbohydrates [152–154].

Nowadays, algal biofuel is suffering from several drawbacks, jeopardising its commercialisation on an industrial scale due to high production cost that is far from being competitive with fossil fuel, and also questioning the sustainability of this production. Hence, different studies considered life cycle assessment analysis as an effective tool to identify the reasons leading to production deficit and exploring its environmental impact [155–162]. Therefore, it was agreed that the major costs come from infrastructure, production set-up, fertilizers, harvesting, drying the biomass, transportation, water footprints, cell disruption and oil extraction process. For instance, Lardon et al. [163] performed an analysis by taking into account all the energetic debt for 1 MJ biodiesel production from *C. vulgaris*. The only positive balance obtained was 0.57 MJ for wet oil extraction with low nitrogen for cell growth (Table 7), and all the other revealed negative balance. Hence, microalgal biofuel production still needs efficient improvement to reduce energy input needed in order to reach competitive prices with petroleum in the market, and more important to be an overall sustainable production.

### 7.2. Human nutrition

*C. vulgaris* is one of the few microalgae that can be found in the market as a food supplement or additive [5,140], colourant (*C. vulgaris* after carotenogenesis) and food emulsion [119]. These products come in different forms such as capsules, tablets, extracts and powder [164,165]. Nevertheless, despite all the healthy benefits that *C. vulgaris* and other microalgae can provide, with their remarkable richness in proteins, lipids, polysaccharides, pigments and vitamins, they are rather considered as nutraceuticals instead of food products due to the lack of clear common official legislations in terms of quality and requirements regarding microalgae [166,167]. Moreover, *C. vulgaris* extract proved to have preservative activity higher than those obtained synthetically, i.e., butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) [168].

### 7.3. Animal feed

It is estimated that about 30% of microalgal production is sold for animal feed purposes [169] due to the increasing demand for

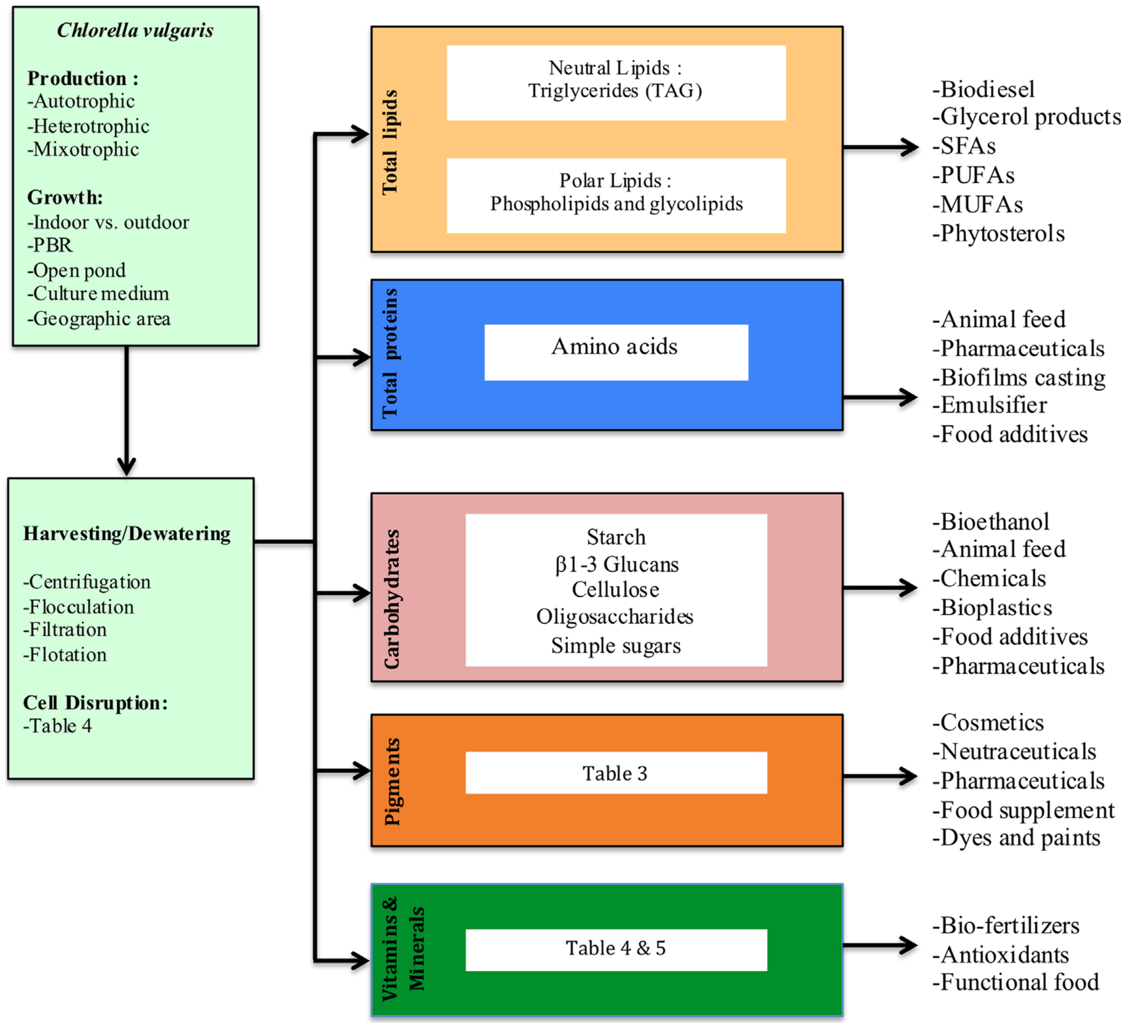



**Fig. 4.** Algo-refinery concept from production to valorisation.

food with natural composition instead of synthesised ingredients. This has triggered intensive research into finding natural ingredients that improve the quality of animal food products [119]. Thus, while stressing *C. vulgaris*, it accumulates important amount of carotenoids and after feeding it to animals such as fish and poultry it showed interesting pigmentation potential for fish flesh and egg yolk in poultry, together with enhancing health and increasing life expectancy of animals [165,169–174]. Moreover, *C. vulgaris* showed a protective effect against heavy metals and other harmful compounds (lead, cadmium, and naphtalene) by reducing significantly the oxidative stress induced by these harmful compounds, and increasing the antioxidant activity in the organisms of tested animals [175–177].

### 7.4. Wastewater treatment

Many studies demonstrated the remarkable potential of *C. vulgaris* in fixating up to 74% carbon dioxide when grown in a photobioreactor [178], and in absorbing 45–97% nitrogen, 28–96% phosphorus and in reducing the chemical oxygen demand (COD) by 61–86% from different type of wastewater such as textile, sewage, municipal, agricultural and recalcitrant [179–185]. Microalgae provide a pathway for the removal of vital nutrients (nitrogen and phosphorus), carbon dioxide, heavy metals and pathogens present in wastewaters and necessary for their growth. In addition, saving and requirements for chemical remediation and possible minimisation of fresh water use for biomass production are the main drivers for growing microalgae as part of a wastewater treatment process [46]. Thus, a faster growth rate accompanied by an elimination of water-contamination level is a promising and advantageous process. Furthermore, performance of *C. vulgaris* in synthesised wastewater was improved when co-immobilised in alginate beads with microalgae growth-promoting bacteria, and removed 100% of ammonium ($NH_4^+$) during four consecutive cycles of 48 h, and 83% for phosphorus after one cycle of 48 h [186]. Thus, *C. vulgaris* is considered as one of the best microalga for bioremediation of wastewater with an impressive potential to completely remove ammonium and sometimes modest potential to eliminate phosphorus present in the medium [187].

### 7.5. Agrochemical applications

Blue-green algal extract excretes a great number of substances that influence plant growth and development [188]. These microorganisms have been reported to benefit plants by producing growth promoting regulators, vitamins, amino acids, polypeptides, antibacterial and antifungal substances that exert phytopathogen biocontrol, and polymers such as exopolysaccharides that improve plant growth and productivity [189].

The bio-fertilisation effect using algae extract are recommended for increasing the growth parameters of many plants [190,191]. This is due to the biochemical profile of algae extract rich in nitrogenase, nitrate reductase, and minerals, which are

essential nutrients for plant growth. The effect of the aqueous extract of *C. vulgaris* as foliar feeding on nutrients status, growth, and yield of wheat plant (*Triticum aestivum* L. var. Giz 69) has been investigated [192]. Thus, this study found that a concentration of 50% (v/v) algae extract as one time foliar spray (25 days after sowing) increased the growth yield and weight gain by 140% and 40%, respectively. Moreover, another study showed the bio-fertilisation impact of *C. vulgaris* on growth parameters and physiological responses of *Lactuca sativa* germination seeds in culture medium containing microalga grown for 3, 6, 9, 12 and 15 days [193]. As a result, the addition of *C. vulgaris* to the culture medium or soil significantly increased fresh and dry weight of seedlings as well as pigments content. The best treatments were 2 and 3 g dry alga $kg^{-1}$ soil. All these studies were conducted on the liquid extract of *C. vulgaris* as bio-fertilizer for plant growth. Therefore, further studies should be carried out to estimate costs on a large scale of the algae cell extract as foliar fertilizer, compared to other commercial foliar fertilizers present in the market.

## 8. Algo-refinery concept

The concept of biorefinery has been inspired from the petroleum refinery concept. It reflects a platform that integrates a process to fractionate the components of a biomass [194,195] to produce multiple products, and thus a biorefinery takes advantage of the various components in the biomass in order to improve the value derived from each component and also generating its own power, which maximises profitability and preserve the environment. Hence, *C. vulgaris* with all its potential and richness in proteins, carbohydrates, lipids, pigments, minerals and vitamins described previously deserves to be completely refined (Fig. 4) without forgetting that every operation unit should take into account the next stage and preserve the integrity of all components of interest in the downstream process.

## 9. Conclusion

This review reflects a broader image about the potential benefits of *C. vulgaris*, and gives an insight about the technological advancements already conducted. *C. vulgaris* can easily be cultured with inexpensive nutrient regime and has faster growth rate as compared to terrestrial energy crops and high biomass productivity. However, production-processing cost remains too high to compete in the market. Indeed, this is the major problem facing the microalgal industry nowadays, but it should be recognised that much improvements have been achieved during the last decade and expectations are estimating that the nearest future of microalgal industry will be strongly competitive on different levels in the market. The remarkable values of *C. vulgaris* set the groundwork to additional research for futuristic applications where it will be represented as a strong candidate for tomorrow's bio-industry.

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